PATENT SPECIFICATION

NO DRAWINGS

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Date of Application and filing Complete Specification May 13, 1957.

No. 15154/57.

Application made in United States of America on May 21, 1956.

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COMPLETE SPECIFICATION

Albumin Diagnostic Composition

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PATENTS ACT, 1949

SPECIFICATION NO. 826,066

Reference has been directed, in pursuance of Section 8, of the Patents Act, 1949, to Specification No. 814,223.

THE PATENT OFFICE,
29th January, 1962

DS 60237/1(1)/R.153 200 1/62 PL

[illegible] most of these [illegible] treated with an albumin precipitant, such as nitric acid, sulphosalicylic acid, picric acid or acetic acid with heat, and the presence of albumin is indicated by the formation of a precipitate, the volume or density of which is dependent on the amount of albumin present.

In all such tests, wherein the precipitation of albumin is an essential step, some laboratory apparatus is required, and the testing procedures are further complicated by the necessity of preparing and handling the corrosive reagents required. Furthermore, when it is desired to conduct a large number of albumin determinations by the aforementioned precipitation tests, the greatly multiplied manipulative steps required by such a series of tests demand the services of a correspondingly large staff of technicians, if the results are to be known within a reasonable time.

It would obviously be of advantage to devise a test for albumin which would not require any specialized apparatus or liquid reagents other than the specimen being tested, and which could be conducted easily and reliably even by unskilled persons. Accordingly, it is an object of the invention to provide an albumin diagnostic composition which can be used by unskilled persons, without special facilities.

The principle underlying the present invention is the phenomenon of "protein error" exhibited by certain indicators whereby, in the presence of proteins, such indicators will undergo their characteristc colour changes at lower pH values than that at which they will change colour in the absence of protein. That is to say, an indicator which exhibits protein error will, in a solution containing protein, by its colour indicate a higher pH value for such solution than is actually the case, the extent to which the characteristic colour-change point of the indicator is shifted is some indication of the amount of protein in the solution.

In accordance with the invention, therefore, an indicator for determining albumin in liquids is provided, comprising a bibulous body carrying an indicator dye which exhibits protein error, and a solid acid-reacting material effective to buffer the body and the dye at a point adjacent to, but on the acid side of, the pH at which the colour change of the dye normally occurs.

This invention also consists in a method of

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COMPLETE SPECIFICATION

Albumin Diagnostic Composition

We, MILES LABORATORIES, INC., a Corporation organized and existing under the laws of the State of Indiana, United States of America, of 1127, Myrtle Street, Elkhart, Indiana, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to an indicator for the detection of albumin in liquids, and relates particularly to indicator bodies or compositions having utility in the qualitative and semi-quantitative determination of albumin in body fluids, such as urine.

The presence of albumin in urine has long been known to be an indication of a diseased or traumatic condition, and many tests for the detection of albumin have been developed. In most of these tests, the urine specimen is treated with an albumin precipitant, such as nitric acid, sulphosalicylic acid, picric acid or acetic acid with heat, and the presence of albumin is indicated by the formation of a precipitate, the volume or density of which is dependent on the amount of albumin present.

In all such tests, wherein the precipitation of albumin is an essential step, some laboratory apparatus is required, and the testing procedures are further complicated by the necessity of preparing and handling the corrosive reagents required. Furthermore, when it is desired to conduct a large number of albumin determinations by the aforementioned precipitation tests, the greatly multiplied manipulative steps required by such a series of tests demand the services of a correspondingly large staff of technicians, if the results are to be known within a reasonable time.

It would obviously be of advantage to devise a test for albumin which would not require any specialized apparatus or liquid reagents other than the specimen being tested, and which could be conducted easily and reliably even by unskilled persons. Accordingly, it is an object of the invention to provide a solid, dry indicator composition, which incorporates all the reagents necessary for determining the presence of albumin in liquids, and which will reduce the manipulative steps required for such determination to the simple act of applying to such composition a small quantity of the liquid to be tested.

An additional object is to provide an indicator of the type referred to in the previous paragraph, which is conveniently fabricated from bibulous material which may take the form of thin absorbent sheet material, such as paper, impregnated with a reagent which, upon contact with the liquid to be tested, will undergo a colour change if albumin is present.

A further object is to provide an indicator body or composition having utility in testing for albumin in urine, which will permit quick and reliable determinations to be made even by unskilled persons, without special facilities.

The principle underlying the present invention is the phenomenon of "protein error" exhibited by certain indicators whereby, in the presence of proteins, such indicators will undergo their characteristc colour changes at lower pH values than that at which they will change colour in the absence of protein. That is to say, an indicator which exhibits protein error will, in a solution containing protein, by its colour indicate a higher pH value for such solution than is actually the case, the extent to which the characteristic colour-change point of the indicator is shifted is some indication of the amount of protein in the solution.

In accordance with the invention, therefore, an indicator for determining albumin in liquids is provided, comprising a bibulous body carrying an indicator dye which exhibits protein error, and a solid acid-reacting material effective to buffer the body and the dye at a point adjacent to, but on the acid side of, the pH at which the colour change of the dye normally occurs.

This invention also consists in a method of

making an indicator for detecting albumin in liquids, which comprises (1) impregnating a bibulous carrier with a solution of an indicator dye which exhibits protein error, and a solid acid-reacting material effective to buffer the carrier and the dye at a point adjacent to, but on the acid side of, the pH at which the colour change of the dye normally occurs and (2) drying the carrier.

When an indicator according to the invention is used to test a liquid specimen, for example, a urine specimen, (i.e., a liquid having a pH of about 4.5 to 8.5), the colour exhibited by the indicator composition prior to an albumin test is, therefore, that colour which is characteristic of the dye at the pH just below the colour-change point; when such a indicator composition is moistened with a liquid to be tested, if the liquid does not contain protein (albumin) the composition does not change colour; on the other hand, if the liquid does contain protein, the protein error of the dye takes effect and a colour characteristic of a somewhat higher pH is formed, which results in a change of colour of the indicator composition.

An indicator composition according to the invention is most conveniently prepared by impregnating a filter paper carrier strip with a solution of the indicator dye and buffer and thereafter air-drying the impregnated carrier. As would be expected, only a small amount of indicator dye is needed to colour the carrier strips adequately in use. We have found, for example, that carrier strips are adequately coloured by wetting them with an impregnating solution containing 0.005% to 0.3%, and preferably 0.01% to 0.07% by weight, of the dye. However, these dye proportions are not critical and somewhat higher or lower proportions may be used if desired.

The proportion of buffer in the impregnating solution, which buffer in our "preferred" embodiments takes the form of solid acids or acid salts, will of course vary with the particular buffer used with the pH value at which it is desired to buffer the carrier and dye. We have found that when urine is the liquid to be tested for albumin, using the buffers hereinafter identified, a carrier strip, which is suitably buffered to any pH which may be required by the usual indicator dyes, may be prepared by wetting the carrier with an impregnating solution containing 0.5% to 25% preferably 1.0% to 8.0%, by weight of the buffer and thereafter drying the carrier. However, buffer concentrations outside that range may of course be employed if necessary.

The following examples are illustrative of the indicator compositions of the invention and of the method of preparing them:—

Example 1

35 mg. of a citrate buffer salt (containing 27% sodium citrate dihydrate and 73% anhydrous citric acid) is dissolved in 0.5 ml. water. To this is added 0.5 ml. of 0.1% tetrabromophenolphthalein ethyl ester dissolved in ethyl alcohol. This mixed solution is used to impregnate strips of filter paper, such as Eaton and Dikeman No. 623. After being air-dried the treated strips are ready for use. Albumin test papers prepared in this way are buffered to about pH 3. Stability tests on indicator compositions prepared in this manner indicate that they are completely stable for at least five months.

Example II

Standard filter paper of medium thickness (about 0.0025 in.) is cut into strips of convenient size which are impregnated with a solution having the following composition, and are then air-dried:—

Brom-phenol blue	0.002 gm.
Phthalic acid	1.000 gm.
Fumaric acid	1.000 gm.
Water	20 ml.

Example III

Strips of filter paper are dipped in a solution having the following composition, and are thereafter air-dried:—

Brom phenol blue	0.002 gm.
Aluminium sulphate	5.000 gm.
Water	20 ml.

Although we prefer to prepare our indicator compositions by impregnating strips of paper with a solution of the dye and buffer agent, as described in the above examples, because of the obvious convenience of that procedure, we intend to include within the scope of our invention indicator compositions prepared by other methods, such as by adhesively fixing to the surface of that carrier a finely-divided dry, intimate mixture of the dye and the solid buffer agent using any suitable protein-free adhesive such as starch.

Our experience leads us to believe that practically any solid acid may be used in a buffer capacity in the present indicator compositions, provided the acid is capable of lowering the pH of the composition below the colour-change point of the indicator dye. For instance, in Example I, wherein the colour-change point of the dye (tetrabromophenolphthalein ethyl ester) is pH 3.5, the following acids (used singly or in combination): tartaric, maleic, ascorbic, salicylic, sulphosalicylic, oxalic, itaconic, gluconic, sulphamic, succinic, benzoic, mandelic, glutaric, malic and phthalic are among those which may be substituted for the citric acid-sodium citrate buffer specifically disclosed.

In Example II, wherein the dye used (Brom phenol blue) has a colour-change point at pH 3.0 aluminium sulphate or aluminium chloride, or suitable formulations including one or more of the following: salicylic, citric, fumaric, phthalic, malic, tartaric, sulphamic,

sulphosalicylic, itaconic, succinic, mandelic, glutaric and benzoic acids, may be substituted for the phthalic acid-fumaric acid buffer specifically disclosed in that formulation. However, it will be understood by those skilled in the art that all of the acids which are capable of use as buffers with a particular dye will not necessarily be used in the same proportions set forth in the above examples, since a smaller quantity of the stronger acids will be needed to buffer the composition to the pH value required by that dye than will be necessary when weaker acids are used for this purpose.

Although the indicator dyes specifically identified in the above examples are presently preferred, since when buffered as illustrated they produce a sharply-defined colour-change in the presence of as little as .01% of albumin in the urine being tested, other dyes may also be used in our compositions, provided such dyes exhibit the phenomenon of "protein error" discussed above. Examples of other suitable dyes are Brom Cresol Green dimethylaminoazobenzene and congo red.

In general, we prefer to use dyes in our indicator compositions which exhibit their normal colour-change at a pH of 7 or less because of the wide variety of solid acids and acid-reacting salts which are available for buffering over a wide pH range below pH 7. However, the invention may be practised using dyes whose characteristic colour-change occurs above pH 7, and in which cases buffers effective in the alkaline range, such as $Na\ CO_3$—$NaH\ CO_3$, $Na\ H_2\ PO_4$—$Na_2\ HPO_4$, or $Na_2\ HPO_4$—$Na_3\ PO_3$, will be used.

The material which we prefer to use as the carrier in our indicator compositions is paper stock, as noted above, since it is inexpensive and readily available in the degree of purity most suitable for that purpose. However, other absorbent materials, including certain textile fabrics, may be used as the carrier component, if desired.

The mode of use of our indicator compositions will now be described, using the composition of Example I for reference. As indicated above, a test strip prepared according to this example is buffered by means of the sodium citrate-citric acid to a pH of about 3, and at this pH value the indicator dye (tetrabromophenolphthalein ethyl ester) is yellow. In conducting the test for albumin, such a strip is placed into the urine and promptly removed, since the pH of the moistened strip must be dominated by the buffer in the composition. For this reason, the testing strip could remain only momentarily in contact with the relatively large volume of the urine being tested, in order to avoid any substantial leaching of the buffer from the carrier. Because of the buffer action, the strip, when removed from the urine being tested, retains its yellow colour in the absence of protein (albumin). However, in the presence of protein in the amount of 0.01% and above the dye with which the strip is impregnated will, in response to the protein error, display a blue colour, the intensity of the colour being an index of the proportion of protein in the urine specimen. The compositions of the examples have a sensitivity to protein in the useful clinical range of 0.01% to over 2.0% of protein.

By the foregoing test procedure, good qualitative determinations, and even semi-quantitative determinations of albumin may be obtained. For more accurate results, the dye colours produced by albumin in the urine being tested are compared with a colour chart showing the colours exhibited by the same dye when subjected to a number of standard solutions of albumin within the range of albumin concentrations found in urine.

It will be seen from the foregoing that the invention in its broadest aspects relates to indicator compositions in the form of convenient carrier compositions or bodies having incorporated therewith an indicator dye which exhibits the phenomenon of "protein error" and a solid buffering agent capable of buffering the carrier and the dye to a pH somewhat below the pH value at which the dye normally undergoes its characteristic colour-change. Accordingly, it is to be understood that the description and examples set forth hereinabove are intended to define and illustrate, but not to limit, the invention to the particular reagents or proportions set forth herein.

WHAT WE CLAIM IS:—

1. An indicator for determining albumin in liquids, comprising a bibulous body carrying an indicator dye which exhibits protein error, and a solid acid-reacting material effective to buffer the body and the dye at a point adjacent to, but on the acid side of, the pH at which the colour change of the dye normally occurs.

2. An indicator as claimed in Claim 1, which comprises a dry bibulous carrier having incorporated therein the solids remaining after impregnation of the carrier with a solution of the indicator dye and the acid-reacting material, the dye being present in the solution in an amount of from 0.005% to 0.30% by weight and the acid being present in the solution in an amount of from 0.5% to 25% by weight.

3. An indicator as claimed in Claim 1 or 2 in which the acid-reacting material is a mixture of sodium citrate and citric acid.

4. An indicator as claimed in Claim 1, 2 or 3, in which the indicator dye is bromphenol blue.

5. An indicator as claimed in Claim 1, 2 or 3, in which the indicator dye is tetrabromophenolphthalein ethyl ester.

6. An indicator as claimed in Claim 2, in which the carrier incorporates the solids remaining after impregnating the carrier with

a solution containing 0.05% by weight of tetrabromophenolphthalein ethyl ester and 3.5% by weight of the mixture of sodium citrate and citric acid, about 27% of the mixture consisting of sodium citrate dihydrate and the remainder consisting of anhydrous citric acid.

7. An indicator as claimed in Claim 2, in which the carrier has incorporated therein the solids remaining after impregnation of the carrier with a solution having the following composition:—

Brom-phenol blue - -	0.002 gm.
Phthalic acid - - -	1.000 gm.
Fumaric acid - - -	1.000 gm.
Water - - - -	20 ml.

8. An indicator as claimed in Claim 2, in which the carrier has incorporated therein the solids remaining after impregnation of the carrier with a solution having the followng composition:—

Brom-phenol blue - -	0.002 gm.
Aluminium sulphate -	5.000 gm.
Water - - - - -	20 ml

9. An indicator for detecting albumin in liquids, substantially as described in the foregoing examples.

10. A method of making an indicator for detecting albumin in liquids, which comprises (1) impregnating a bibulous carrier with a solution of an indicator dye which exhibits protein error, and a solid acid-reacting material effective to buffer the carrier and the dye at a point adjacent to, but on the acid side of, the pH at which the colour change of the dye normally occurs and (2) drying the carrier.

11. A method of making an indicator for detecting albumin in liquids, substantially as described with reference to the foregoing examples.

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